PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : **A61K 39/39 // C08L 101/02, 33/02, 41/02, A61K 39/71, 39/145**	**A2**	(11) International Publication Number: **WO 98/17310** (43) International Publication Date: 30 April 1998 (30.04.98)

(21) International Application Number: PCT/EP97/05861

(22) International Filing Date: 23 October 1997 (23.10.97)

(30) Priority Data:
9622159.3 24 October 1996 (24.10.96) GB

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: POLYANIONIC POLYMERS AS ADJUVANTS FOR MUCOSAL IMMUNIZATION

(57) Abstract

Mucosal adjuvants for vaccines are disclosed comprised of water–soluble polyanionic polymers which have anionic constitutional units obtained from acids such as acrylic acid, methacrylic acid, maleic acid, fumaric acid, ethylsulphonic acid, vinylsulphuric acid, vinylsulphonic acid, styrenesulphonic acid, vinylphenylsulphuric acid, 2–methacryloyloxyethane sulphonic acid, 3–methacryloyloxy–2–hydroxypropanesulphonic acid, 3–methacryl amido–3–methylbutanoic acid, acrylamidomethylpropanesulphonic acid, vinylphosphoric acid, 4–vinylbenzoic acid, 3–vinyl oxypropane–1–sulphonic acid, N–vinylsuccinimidic acid, and salts of the foregoing. The polyanionic polymers may further have hydrophobic constitutional repeating units, such as alkylesters, cycloalkylesters, hydroxyalkylesters, ethers, glycols, aromatic groups and salts thereof. Also disclosed herein are use of the polyanionic polymers of the present invention for the induction or enhancement of mucosal immune responses, as well as nonparenteral vaccines containing the polyanionic polymer of the present invention.

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against respiratory diseases, gastro-intestinal diseases and sexually-transmissible diseases) which are safe, inexpensive, easy to produce and easy to incorporate in to such mucosal vaccines in which they are to be employed.

It is a still further primary object of the present invention to provide mucosal vaccines which incorporate such mucosal adjuvants therein for inducing or enhancing immune responses to antigens.

It is a yet further primary object of the present invention to provide methods for inducing or enhancing immune responses to antigens and to provide methods for providing mucosal adjuvants and mucosal vaccines comprised of mucosal adjuvants which are capable of such inducement or enhancement.

The present invention relates to mucosal adjuvants for incorporation into mucosal vaccines and to mucosal vaccines incorporating such adjuvants therein useful for the induction or enhancement of mucosal and/or systemic immune responses to antigens.

Thus, in accordance with the teachings of the present invention, there is provided a mucosal adjuvant for vaccines comprising a water-soluble polyanionic polymer having anionic constitutional repeating units.

The mucosal adjuvants of the present invention are water-soluble polyanionic polymers having anionic constitutional repeating units which may be the same or different repeating units, or polyanionic polymers having anionic constitutional repeating units (same or different) and hydrophobic constitutional repeating units. The polyanionic polymers may be linear (polymers having chemical units which are connected covalently to one or two other constitutional units), or branched (polymers having chemical units which are connected covalently to one or two other constitutional units and occasionally to three or more constitutional units) or reticular (polymers having chemical units which are connected covalently to one or two or three or more other constitutional units) in structure.

As used herein, the following terms have the following meanings :

The term "water soluble", when referring to the polyanionic polymers of the present invention refers to polymers which are soluble in an aqueous phase at a concentration of at least 0.01 gram per liter.

The term "polymer refers to compounds having at least three identical chemical constitutional repeating units, which said units are covalently connected with one another.

The term "constitutional repeating unit refers to the minimal structural unit of a polymer.

The term "homopolymer" refers to polymers consisting of one type of constitutional repeating unit.

The term "heteropolymer" refers to polymers having two or more different constitutional repeating units.

The term "polyanionic polymer" refers to polymers which, when dissolved in an aqueous medium, are negatively charged due to the presence of anionic constitutional repeating units (e.g., units containing sulphate, sulphonate, carboxylate, phosphate and borate groups).

The term "anionic constitutional repeating unit" refers to constitutional repeating units of polymers which are negatively-charged in aqueous medium at physiological conditions.

The term "hydrophobic constitutional repeating unit" refers to constitutional repeating units of polymers which are characterised in that the corresponding monomer is less soluble in an aqueous phase than in an organic solvent [that is to say, the quantity, in weight (in grams), of the monomer that can be dissolved in a fixed volume, in ml, of an aqueous phase is less than the quantity, in weight (in grams), of the monomer that can be dissolved in the same fixed volume, in ml, of the organic solvent].

In the mucosal adjuvants of the present invention the anionic constitutional repeating units of the polyanionic polymer are preferably obtained from acrylic acid, methacrylic acid, maleic acid, fumaric acid, ethylsulphonic acid, vinylsulphuric acid, vinylsulphonic acid, styrenesulphonic acid (vinylbenzenesulphonic acid), vinylphenylsulphuric acid, 2-methacryloyloxyethane sulphonic acid, 3-methacryloyloxy-2-hydroxypropanesulphonic acid, 3-methacryl amido-3-methylbutanoic acid, acrylamidomethylpropanesulfonic acid, vinylphosphoric acid, 4-vinylbenzoic acid, 3-vinyl oxypropane-1-sulphonic acid, N-vinylsuccinimidic acid, and salts of the foregoing.

More preferably, in the mucosal adjuvants of the present invention, the anionic constitutional repeating units of the polyanionic polymer are obtained from acrylic acid, methacrylic acid, maleic acid, fumaric acid, ethylsulphonic acid, vinylsulphuric acid, vinylsulphonic acid, styrenesulphonic acid, and acrylamidomethylpropanesulfonic acid, and salts of the foregoing.

Most preferred in the mucosal adjuvants of the present invention, the anionic constitutional repeating units of the polyanionic polymer are obtained from acrylic acid, methacrylic acid, maleic acid and fumaric acid, vinylsulphonic acid, styrenesulphonic acid, and acrylamidomethylpropanesulfonic acid, and salts of the foregoing.

The mucosal adjuvants of the present invention include polyanionic homopolymers which are preferably obtained from polyacrylic acid, polymethacrylic acid, polymaleic acid, polyfumaric acid, polyethylsulphonic acid, polyvinylsulphuric acid, polyvinylsulphonic acid, polystyrenesulphonic acid (polyvinylbenzenesulphonic acid), polyvinylphenylsulphuric acid, poly 2-methacryloyloxyethanesulphonic acid, poly

3-methacryloyloxy-2-hydroxypropanesulphonic acid, poly 3-methacryl amido-3-methylbutanoic acid, polyacrylamidomethylpropanesulfonic acid, polyvinylphosphoric acid, poly 4-vinylbenzoic acid, poly 3-vinyl oxypropane-1-sulphonic acid, poly N-vinylsuccinimidic acid, and salts of the foregoing.

More preferably, the polyanionic homopolymers are obtained from polyacrylic acid, polymethacrylic acid, polymaleic acid, polyfumaric acid, and salts of any of the fore going.

The mucosal adjuvants of the present invention further include polyanionic heteropolymers having two different (distinct) anionic groups, such as, but not limited to, a carboxylic group and a sulfate or sulfonic group, for example, acrylic acid and any of vinylsulphonic acid, styrenesulphonic acid and acrylamidomethylpropanesulfonic acid.

In a preferred embodiment, the polyanionic polymer of the mucosal adjuvants of the present invention further has hydrophobic constitutional repeating units.

The hydrophobic constitutional repeating units of the polyanionic polymer of the mucosal adjuvants of the present invention are obtained from alkylesters, cycloalkylesters, hydroxyalkylesters, ethers, glycols and aromatic groups and salts of the foregoing.

Preferably, in the mucosal adjuvants of the present invention, the alkylesters are selected from the group consisting of methyl-, ethyl-, propyl-, isopropyl, n-butyl-, isobutyl, sec.butyl-, t-butyl, n-hexyl-, n-octyl-, isooctyl-, 2-ethylhexyl-, n-decyl-, tetradecyl-, vinyl-, allyl- and oleylester.

Preferably, in the mucosal adjuvants of the present invention, the cycloalkylesters are selected from the group consisting of cyclohexyl-, 1-methylcyclohexyl-, 3-vinylcyclohexyl- and 3,3,5-trimethylcyclohexylester.

Preferably, in the mucosal adjuvants of the present invention, the hydroxyalkylesters are selected from the group consisting of 2-hydroxyethyl-, 2-hydroxypropyl-, 3-hydroxypropyl-, 3,4-dihydroxybutyl-, 2-hydroxypenyl- and 2-hydroxyhexylester.

Preferably, in the mucosal adjuvants of the present invention the ethers are selected from the from the group consisting of methoxymethyl, ethoxyethyl, allyloxymethyl, 2-ethoxyethoxymethyl, benzyloxymethyl, cyclohexyloxymethyl, 1-ethoxyethyl, 2-ethoxyethyl, 2-butoxyethyl, methoxymethoxyethyl, methoxyethoxyethyl, 1-butoxypropyl, 1-ethoxybutyl, tetrahydrofurfuryl, furfuryl.

Preferably, in the mucosal adjuvants of the present invention, the glycols are selected from the group consisting of ethylene glycol, 1,2-propanediol, 1,3-propanediol, 1,3-butanediol, 1,4-butanediol, 2,5-dimethyl-1,6-hexanediol, 1,10-decanediol, diethyleneglycol and triethyleneglycol.

Preferably, in the mucosal adjuvants of the present invention, the aromatic groups are selected from the group consisting of benzyl, phenyl and nonylphenyl.

More preferably, in the mucosal adjuvants of the present invention the hydrophobic constitutional repeating units of the polyanionic polymer are obtained from the group consisting of methyl-, ethyl, propyl, butyl-, pentyl-, hexyl-, heptyl-, octyl-, nonyl-, and decyl- esters of acrylic acid, methacrylic acid, maleic acid, fumaric acid, ethylsulphonic acid, vinylsulphuric acid and styrenesulphonic acid and salts of the foregoing.

Most preferably, in the mucosal adjuvants of the present invention the hydrophobic constitutional repeating units of the polyanionic polymer are obtained from the group consisting of butyl-, pentyl-, hexyl-, heptyl- and octyl- esters of acrylic acid, methacrylic acid, maleic acid and fumaric acid and salts of the foregoing.

Particulaly preferred polyanionic polymers according to the present invention are polyacrylic acid, butyl-polyacrylic acid, poly(acrylate-co-acrylamidomethylpropane sulfonic acid) copolymer (p(A-c-AMPS)), poly(acrylate-co-vinylsulfonate) copolymer (p(A-c-VS)), poly(acrylate-co-vinylbenzenesulfonate) copolymer (p(A-c-VBS)),

It is further preferred that in the mucosal adjuvants of the present invention, the molar ratio of hydrophobic constitutional repeating units and anionic constitutional repeating units of the polyanionic polymers of the present invention is between 0 hydrophobic constitutional repeating units per 1 anionic constitutional repeating unit and 0.6 hydrophobic constitutional repeating units per 1 anionic constitutional repeating unit.

More preferably, in the mucosal adjuvants of the present invention, the molar ratio of hydrophobic constitutional repeating units and anionic constitutional repeating units is between 0.02 and 0.60 hydrophobic constitutional repeating unit per 1 anionic constitutional repeating unit (which is from 2 to 60 hydrophobic constitutional repeating units per every 100 anionic constitutional repeating units).

Most preferably, in the mucosal adjuvants of the present invention, the molar ratio of hydrophobic constitutional repeating units and anionic constitutional repeating units is between 0.05 and 0.30 hydrophobic constitutional repeating units per 1 anionic constitutional repeating unit (which is from 5 to 30 hydrophobic constitutional repeating units per every 100 anionic constitutional repeating units).

In another aspect of the present invention, disclosed herein is a mucosal (nonparenteral) vaccine having the mucosal adjuvant (including the polyanionic polymer thereof) of the present invention, wherein the vaccine is administered nonparenterally for the induction of either systemic or mucosal immunity against antigens.

In this aspect, the mucosal vaccine may further be comprised of an antigen or a drug molecule and/or a pharmaceutically-acceptable medium (carrier).